厚生労働科学研究費補助金

がん臨床研究事業

未治療原発不明癌に対するDNAチップを用いた原発巣推定に基づく治療効果の意義を問う無作為化第Ⅱ相試験

平成25年度　総括研究報告書

研究代表者　中　川　和　彦

平成26（2014）年　3月

様式Ａ（８）

厚生労働科学研究費補助金研究報告書

平成 26 年 3 月 31 日

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平成 25 年度厚生労働科学研究費補助金（　がん臨床　研究事業）に係る研究事業を完了したので次のとおり報告する。

研究課題名（課題番号）：　未治療原発不明癌に対するDNAチップを用いた原発巣推定に基づく治療効果の意義を問う無作為化第II相試験（　H25-がん臨床-一般-010　）

国庫補助金精算所要額　：金　２４，３００，０００　円也（うち間接経費　５，６０７，０００円）

１．厚生労働科学研究費補助金研究報告書表紙　（別添１のとおり）

２．厚生労働科学研究費補助金研究報告書目次　（別添２のとおり）

３．厚生労働科学研究費補助金総括研究報告書　（別添３のとおり）

４．研究成果の刊行に関する一覧表　（別添５のとおり）

別添１

厚生労働科学研究費補助金研究報告書表紙

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厚生労働科学研究費補助金研究報告書目次

別添３

厚生労働科学研究費補助金総括研究報告書

別添５

研究成果の刊行に関する一覧表

別紙１

厚生労働科学研究費補助金

がん臨床研究事業

未治療原発不明癌に対するDNAチップを用いた原発巣推定に基づく治療効果の意義を問う無作為化第II相試験

平成２５年度　総括研究報告書

研究代表者　中川　和彦

平成２６（２０１４）年　３月

別紙２

目　　次

別紙３

厚生労働科学研究費補助金（がん臨床研究事業）
総括研究報告書

未治療原発不明癌に対するDNAチップを用いた原発巣推定に基づく治療効果の意義を問う無作為化第II相試験

研究代表者　中川　和彦
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研究要旨　原発不明がん(CUP)を対象とした遺伝子発現解析により原発巣の推定を行う新しい治療戦略の、従来のCUP治療戦略に対する臨床的有用性を問う第III相比較試験の実施妥当性を無作為化臨床第II相試験にて評価する。その際に得られたCUPにおける遺伝子発現プロフィールを用い、より精度の高いCUP診断薬およびCUP特異的分子標的薬の創生を目指す。

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Ａ．研究目的

原発不明がん(CUP)を対象とした遺伝子発現解析により原発巣の推定を行う新しい治療戦略の、従来のCUP治療戦略に対する臨床的有用性を問う第III相比較試験の実施妥当性を無作為化臨床第II相試験にて評価する。その際に得られたCUPにおける遺伝子発現プロフィールを用い、より精度の高いCUP診断薬およびCUP特異的分子標的薬の創生を目指す。

Ｂ．研究方法

１．臨床試験を継続、割り付けおよび治療が実施された103例の登録が現在まで得られているが、当初の目標症例120例を完遂する。
登録、検体採取、原発巣の推定、無作為化、治療に至る複雑な過程を有しているが、現在までの登録の経験より各施設に周知されるに至っており継続とする。
2．最終症例の登録から2年が経過した時点でデータセンターは最終解析を行い、「最終解析報告書」を研究事務局に提出する。
3．本臨床試験において付随的に得られた遺伝子発現プロフィールを用いた付随研究として、以下のトランスレーショナル研究を実施する。
①CUPに特徴的な分子の生物学的意義の検討：現在までの解析結果では、個々の原発巣推定に有用な遺伝子セットが選択されている。一方、興味深いことに「CUPに特徴的」な遺伝子群も同定されており、その中には上皮間葉移行(EMT)に関連する遺伝子等が含まれており原発不明癌の転移を主体とする生物学的特徴を反映する結果を得られつつある。CUPに特徴的な遺伝子群は、未知のCUPの生物学的特徴を反映し、治療戦略への応用が可能となると考えられるため、in vivo転移モデルでの機能解析を含めた基礎研究を行う。
②CUP診断キットの開発：我々の開発した原発巣推定アルゴリズムは、大量の固形癌の遺伝子発現データを基に多施設共同臨床試験症例のデータを数十例使用して改善・機能向上を続けて進化してきた。本研究においては、残りの60例の原発巣推定能力を検証することおよび臨床検体の組織（胸水、リンパ節、転移巣）の遺伝子発現バックグラウンドを考慮した機能向上を目指す。DEFINEは、現在エクセルベースの自動で原発巣推定結果を示すソフトウェアであるが、例えばribosomal proteinなど原発巣推定アルゴリズムで使用した遺伝子群をさらに絞り込み20個前後のリアルタイムＰＣＲベースの遺伝子発現値によって原発巣が特定できる診断キットの開発を目指す。すなわち体外診断薬としての承認を得るべく、収集したサンプルによるレトロスペクティブな検証を経て、先進医療申請などのための論文化、臨床的有用性の評価などをすすめ申請に向けて努力する。

（倫理面への配慮）
本研究では、抗癌剤感受性の高い予後良好な原発不明がん患者が本研究から最大限除外されるよう配慮する。さらに、ヘルシンキ宣言およびわが国の「臨床研究に関する倫理指針」に従い、以下の事項を厳守する。
①　研究実施計画書をWJOGプロトコール審査委員会で審査し、各施設のIRB承認の得られた施設のみ症例登録を可能とする。
②　全ての患者に説明文書を用いて十分な説明を行い、考慮の時間を設けた後に患者自身の自由意志による同意を文書で取得する。
③　データの取り扱いに関して、直接個人を識別できる情報を用いず、データベースのセキュリティを確保し、個人情報の保護を厳守する。
④　プロトコール審査委員会、効果・安全性評価委員会を組織し、研究の第三者的監視を行う。

解析でおこなうマイクロアレイによる遺伝子発現解析はヒトゲノム・遺伝子解析研究に関する倫理指針の対象ではないが、指針の趣旨を尊重し、準じた管理を行うことにより個人情報等倫理的に十分に配慮する。

C．研究結果

ＣＵＰ60症例の発現プロファイルをもとに、正常リンパよりもＣＵＰで発現が大きく亢進している44遺伝子のなかから、ＣＵＰの特徴である”転移能”を付与するような遺伝子の探索を試みた。まず、①特定のがん種だけに高発現が見られない遺伝子、②ＣＵＰの全症例のうち半数以上に高発現が起こっている遺伝子、③ＣＵＰのモデル細胞として使った、転移能の優れた非小細胞肺癌株であるA549細胞に高発現する遺伝子、という３つの判定基準をもとに候補遺伝子を２３個に絞り、さらに多孔性フィルターを使ってmigration assayを行った。A549細胞は２４時間で多孔性フィルターの反対側に遊走するが、siRNAによるノックダウン法で、細胞増殖能は変わらず、遊走能のみが大きく損なわれる遺伝子をスクリーニングしたところ、*PRG1*、*MIF*, *S1004A*, *SERF2*, *OAZ1*, *TIMP1*の６個の候補遺伝子が得られた。

次に、このうちPRG1とMIFのshRNAを発現し、これらの遺伝子（およびタンパク）の発現を恒常的に抑制するA549細胞株を樹立し、vivoの実験（マウス左足底への皮下注射）を行った。shRNAの発現用のベクターは同時にGFPの遺伝子も搭載しており、これらの細胞株のマウスにおける増殖を蛍光でモニターすることが可能である。２０日後、実際にがんで膨れた足底に励起光を当てると、その部分（原発巣）に強い蛍光が生じた。また、膝窩リンパ節に相当する部分も弱いながら蛍光を発しており、原発巣からの転移が示唆された。コントロール、PRGおよびMIFのshRNAを発現するA549を各群１０匹ずつ注射したマウスから摘出した膝窩リンパ節は、注射をしていない右足側のリンパ節と比べて肥大していた。

原発巣からリンパ節転移を起こす細胞の割合は、リンパ節における転移がん細胞が発する蛍光量をimageJをもちいて求め、原発巣である足底の蛍光量との比より評価した。その結果、コントロールに比べて、PRGやMIFのノックアウト（shRNA）がリンパ節転移を有意に阻害していることがわかった。

D．考察

転移したがん細胞のリンパ節に占める割合は一様ではないことから、肥大したリンパ節の大きさを同時期に測定するだけでは、各細胞株の転移能を比較することはできない。個々の遺伝子がどれほど転移に関与するかを評価するには、本実験のようなin vivoの系をもちい、足底（原発巣）およびリンパ節（転移巣）の相対蛍光量を測定する方法により可能となると考えられる。

E．結論

PRGやMIFのノックアウト（shRNA）がリンパ節転移を有意に阻害することから、これらはCUPの特徴といえる“転移能”を付与する遺伝子としてCUPの病態に関連していることが示唆された。現在、同様の方法で残りの候補遺伝子（*S1004A, SERF2, OAZ1, TIMP1*）の解析を進めている。現在、がんのバイオロジー（原発組織の生物学的特徴を持つ遺伝子）から抽出した各がん種特異的遺伝子（100遺伝子）を選択し、次世代シークエンサーを用いた高感度な原発巣推定のための新診断キットの開発を行っている。また、MIFに対する阻害剤であるresveratrolの誘導体の評価を進めており、今後CUP特異的分子標的薬の非臨床試験を展開していく予定である。

F．研究発表

論文発表

1. Yang JC, Wu YL, Chan V, Kurnianda J, Nakagawa K, Saijo N, Fukuoka M, McWalter G, McCormack R, Mok TS. Epidermal growth factor receptor mutation analysis in previously unanalyzed histology samples and cytology samples from the phase III Iressa Pan-ASia Study (IPASS). Lung Cancer, S0169-5002(13):00535-00537, 2013.
2. ○Tsuya A, Kurata T, Tamiya A, Okamoto I, Ueda S, Sakai D, Sugimoto N, Matsumoto K, Goto I, Yamamoto N, Fukuoka M, Nakagawa K. A phase II study of cisplatin /S-1 in patients with carcinomas of unknown primary site. Invest New Drugs, 31(6):1568-1572, 2013.
3. Kawakami H, Okamoto I, Okamoto W, Takeda M, Ueda S, Kudo T, Nishina S, Fujisaka Y, Miyazaki M, Tsurutani J, Kurata T, Nakagawa K. Practical Use of Gemcitabine and Cisplatin Combination Therapy as First-Line Treatment for Japanese Patients with Advanced Biliary Tract Cancer. Journal of Cancer Therapy, 4:1068-1073, 2013.
4. Shaw AT, Kim DW, Nakagawa K, Seto T, Crinó L, Ahn MJ, De Pas T, Besse B, Solomon BJ, Blackhall F, Wu YL, Thomas M, O'Byrne KJ, Moro-Sibilot D, Camidge DR, Mok T, Hirsh V, Riely GJ, Iyer S, Tassell V, Polli A, Wilner KD, Jänne PA. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med, 368(25):2385-94, 2013.
5. Seto T, Kiura K, Nishio M, Nakagawa K, Maemondo M, Inoue A, Hida T, Yamamoto N, Yoshioka H, Harada M, Ohe Y, Nogami N, Takeuch

i K, Shimada T, Tanaka T, Tamura T. CH5424802 (RO5424802) for patients with ALK-rearranged advanced non-small-cell lung cancer (AF-001JP study): a single-arm, open-label, phase 1-2 study. Lancet Oncol, 14(7):590-598, 2013.

6. Yoshioka H, Okamoto I, Morita S, Ando M, Takeda K, Seto T, Yamamoto N, Saka H, Atagi S, Hirashima T, Kudoh S, Satouchi M, Ikeda N, Iwamoto Y, Sawa T, Nakanishi Y, Nakagawa K. Efficacy and safety analysis according to histology for S-1 in combination with carboplatin as first-line chemotherapy in patients with advanced non-small-cell lung cancer: updated results of the West Japan Oncology Group LETS study. Ann Oncol, 24(5):1326-1331, 2013.
7. ○Kurahashi I, Fujita Y, Arao T, Kurata T, Koh Y, Sakai K, Matsumoto K, Tanioka M, Takeda K, Takiguchi Y, Yamamoto N, Tsuya A, Matsubara N, Mukai H, Minami H, Chayahara N, Yamanaka Y, Miwa K, Takahashi S, Takahashi S, Nakagawa K, Nishio K. A microarray-based gene expression analysis to identify diagnostic biomarkers for unknown primary cancer. PLoS One, 8(5):e63249, 2013.
8. Ogi S, Fujita H, Kashihara M, Yamamoto C, Sonoda K, Okamoto I, Nakagawa K, Ohdo S, Tanaka Y, Kuwano M, Ono M. Sorting nexin 2-mediated membrane trafficking of c-Met contributes to sensitivity of molecular targeted drugs. Cancer Science, 104(5):573-5783, 2013.
9. Kawakami H, Okamoto I, Arao T, Okamoto W, Matsumoto K, Taniguchi H, Kuwata K, Yamaguchi H, Nishio K, Nakagawa K, Yamada Y. MET amplification as a potential therapeutic target in gastric cancer. Oncotarget, 4(1):9-17, 2013.
10. Hayashi H, Tsurutani J, Satoh T, Masuda N, Okamoto W, Morinaga R, Terashima M, Miyazaki M, Okamoto I, Nishida Y, Tominaga S, Tokunaga Y, Yamaguchi M, Sakamoto J, Nakayama T, Nakagawa K. Phase II study of bi-weekly irinotecan for patients with previously treated HER2-negative metastatic breast cancer: KMBOG0610B. Breast Cancer, 20(2):131-136, 2013.

G. 知的財産権の出願・登録状況

1. 特許取得　なし
2. 実用新案登録　なし
3. その他　　なし

別紙４

研究成果の刊行に関する一覧表レイアウト

雑誌

発表者氏名	論文タイトル名	発表誌名	出版年	巻号	ページ
Yang JC, Wu YL, Chan V, Kurnianda J, Nakagawa K, Saijo N, Fukuoka M, McWalter G, McCormack R, Mok TS.	Epidermal growth factor receptor mutation analysis in previously unanalyzed histology samples and cytology samples from the phase III Iressa Pan-ASia Study (IPASS).	Lung Cancer.	2013	S0169-5002(13)	00535-00537
○Tsuya A, Kurata T, Tamiya A, Okamoto I, Ueda S, Sakai D, Sugimoto N, Matsumoto K, Goto I, Yamamoto N, Fukuoka M, Nakagawa K.	A phase II study of cisplatin /S-1 in patients with carcinomas of unknown primary site.	Invest New Drugs.	2013	31(6)	1568-1572
Kawakami H, Okamoto I, Okamoto W, Takeda M, Ueda S, Kudo T, Nishina S, Fujisaka Y, Miyazaki M, Tsurutani J, Kurata T, Nakagawa K.	Practical Use of Gemcitabine and Cisplatin Combination Therapy as First-Line Treatment for Japanese Patients with Advanced Biliary Tract Cancer.	Journal of Cancer Therapy.	2013	4	1068-1073
Shaw AT, Kim DW, Nakagawa K, Seto T, Crinó L, Ahn MJ, De Pas T, Besse B, Solomon BJ, Blackhall F, Wu YL, Thomas M, O'Byrne KJ, Moro-Sibilot D, Camidge DR, Mok T, Hirsh V, Riely GJ, Iyer S, Tassell V, Polli A, Wilner KD, Jänne PA.	Crizotinib versus chemotherapy in advanced ALK-positive lung cancer.	N Engl J Med.	2013	368(25)	2385-2394
Seto T, Kiura K, Nishio M, Nakagawa K, Maemondo M, Inoue A, Hida T, Yamamoto N, Yoshioka H, Harada M, Ohe Y, Nogami N, Takeuchi K, Shimada T, Tanaka T, Tamura T.	CH5424802 (RO5424802) for patients with ALK-rearranged advanced non-small-cell lung cancer (AF-001JP study): a single-arm, open-label, phase 1-2 study.	Lancet Oncol.	2013	14(7)	590-598
Yoshioka H, Okamoto I, Morita S, Ando M, Takeda K, Seto T, Yamamoto N, Saka H, Atagi S, Hirashima T, Kudoh S, Satouchi M, Ikeda N, Iwamoto Y, Sawa T, Nakanishi Y, Nakagawa K.	Efficacy and safety analysis according to histology for S-1 in combination with carboplatin as first-line chemotherapy in patients with advanced non-small-cell lung cancer: updated results of the West Japan Oncology Group LETS study.	Ann Oncol.	2013	24(5)	1326-1331
○Kurahashi I, Fujita Y, Arao T, Kurata T, Koh Y, Sakai K, Matsumoto K, Tanioka M, Takeda K, Takiguchi Y, Yamamoto N, Tsuya A, Matsubara N, Mukai H, Minami H, Chayahara N, Yamanaka Y, Miwa K, Takahashi S, Takahashi S, Nakagawa K, Nishio K.	A microarray-based gene expression analysis to identify diagnostic biomarkers for unknown primary cancer.	PLoS One.	2013	8(5)	e63249
Ogi S, Fujita H, Kashihara M, Yamamoto C, Sonoda K, Okamoto I, Nakagawa K, Ohdo S, Tanaka Y, Kuwano M, Ono M.	Sorting nexin 2-mediated membrane trafficking of c-Met contributes to sensitivity of molecular targeted drugs.	Cancer Science	2013	104(5)	573-583
Kawakami H, Okamoto I, Arao T, Okamoto W, Matsumoto K, Taniguchi H, Kuwata K, Yamaguchi H, Nishio K, Nakagawa K, Yamada Y.	MET amplification as a potential therapeutic target in gastric cancer.	Oncotarget.	2013	4(1)	9-17
Hayashi H, Tsurutani J, Satoh T, Masuda N, Okamoto W, Morinaga R, Terashima M, Miyazaki M, Okamoto I, Nishida Y, Tominaga S, Tokunaga Y, Yamaguchi M, Sakamoto J, Nakayama T, Nakagawa K.	Phase II study of bi-weekly irinotecan for patients with previously treated HER2-negative metastatic breast cancer: KMBOG0610B.	Breast Cancer.	2013	20(2)	131-136

Lung Cancer xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Epidermal growth factor receptor mutation analysis in previously unanalyzed histology samples and cytology samples from the phase III Iressa Pan-ASia Study (IPASS)☆,☆☆

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ARTICLE INFO

Article history:
Received 8 November 2013
Accepted 23 November 2013

Keywords:
Cytology
Diagnostic
EGFR
Mutation
Tumor
Histology
NSCLC

ABSTRACT

Objectives: Epidermal growth factor receptor (*EGFR*) mutation testing is standard practice after lung adenocarcinoma diagnosis, and provision of high-quality tumor tissue is ideal. However, there are knowledge gaps regarding the utility of cytology or low tumor content histology samples to establish *EGFR* mutation status, particularly with regard to the proportion of testing performed using these sample types, and the lack of an established link with efficacy of treatment.
Methods: The randomized phase III Iressa Pan-ASia Study (IPASS; ClinicalTrials.gov identifier NCT00322452) of first-line gefitinib versus chemotherapy analyzed samples meeting preplanned specifications ($n = 437$ evaluable for *EGFR* mutation; $n = 261$ mutation-positive). This supplementary analysis assessed tumor content and mutation status of histology ($n = 99$) and cytology samples ($n = 116$) which were previously unanalyzed due to sample quality, type, and tumor content (<100 cells). Objective response rate (ORR) and change in tumor size with gefitinib treatment were assessed.
Results: EGFR mutation testing was successful in 80% and 19% of previously unanalyzed histology and cytology samples, respectively. Mutations were detected in 54 tumors previously described as mutation-unknown (histology, $n = 45$; cytology, $n = 9$). ORRs in mutation-positive cytology (83%) and histology (74%) subgroups were consistent with previous analyses (71%). Tumor size decrease was consistent across previously analyzed and unanalyzed samples (all mutation subgroups), with less consistency across ORRs in mutation-negative cytology (16%) and histology (25%) subgroups versus the previous analysis (1%).
Conclusions: Histology samples with low tumor content and cytology samples can be used for *EGFR* mutation testing; patients whose mutation status was confirmed using these sample types achieved a response to treatment consistent with those confirmed using high-quality histology samples. Better sample quantity/quality can potentially reduce false-negative results.

1. Introduction

Non-small-cell lung cancer (NSCLC) is traditionally treated using platinum-based chemotherapy [1,2]. Recently, the management of advanced lung adenocarcinoma has evolved, and use of molecular diagnosis to investigate driver mutations in tumor samples has become the most important step toward selecting the right agent for a patient's treatment [3].

The most established example is the use of epidermal growth factor receptor (*EGFR*) mutations as a predictive marker of tumor

☆ ClinicalTrials.gov identifier: NCT00322452.

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http://dx.doi.org/10.1016/j.lungcan.2013.11.021

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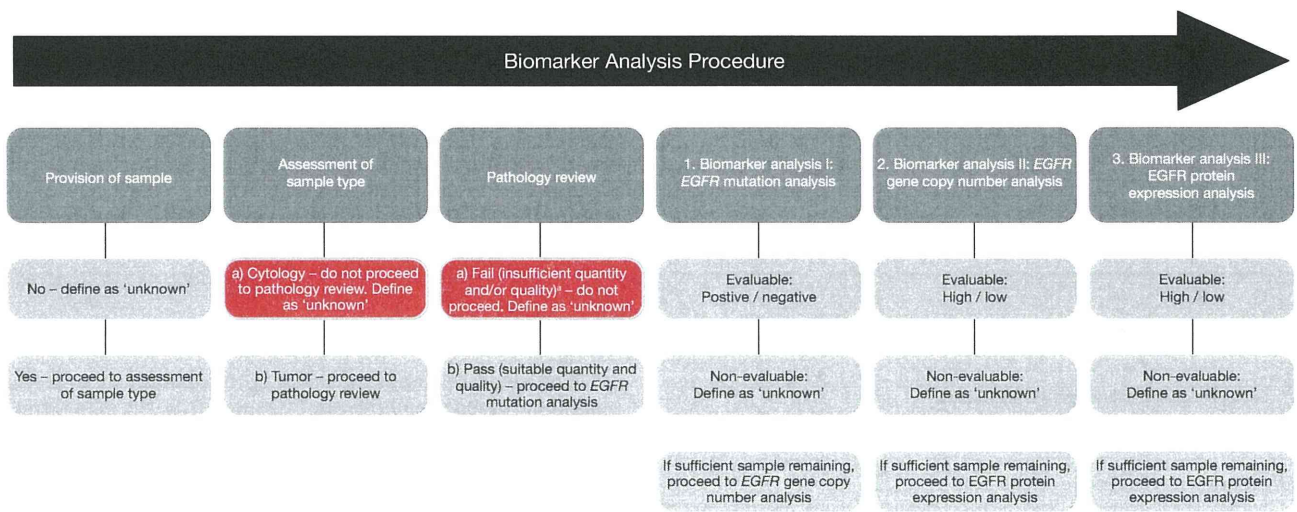
response to EGFR tyrosine kinase inhibitor (TKI) treatment. The first trial to confirm the utility of *EGFR* mutation as a predictor of anticancer efficacy was the Iressa Pan-ASia Study (IPASS), which investigated the outcomes of the overall study population ($n = 1217$) and subgroups (including those evaluable for *EGFR* mutation status [$n = 437$]) treated with gefitinib or carboplatin/paclitaxel [4,5]. IPASS demonstrated superior progression-free survival (PFS), objective response rate (ORR), symptom control, and quality of life with first-line gefitinib versus carboplatin/paclitaxel in patients with *EGFR* mutation-positive tumors. This finding was replicated in the smaller FIRST-Signal study [6]. Five additional phase III studies have subsequently reported significantly increased PFS with EGFR-TKIs (gefitinib, erlotinib, and afatinib) versus platinum-based chemotherapy in patients with *EGFR* mutation-positive tumors [7–11].

IPASS (overall population $n = 1217$) included exploratory objectives to investigate efficacy according to *EGFR* biomarker status (*EGFR* mutation, gene copy number, and protein expression) [4,5]. Collection of histology samples for biomarker analysis was not mandated; 85% of patients consented to donate their tumor. Samples were provided by 683/1217 patients (56%). Fukuoka et al. presented the IPASS exploratory biomarker data for 261 patients with *EGFR* mutation-positive tumors out of 437 evaluable patients (60%) [4].

The streamlined biomarker analysis process (Fig. 1) required all samples to meet stringent pre-specified thresholds for the number of tumor cells and sample quality/type, based on the higher cell requirements of fluorescent in situ hybridization (FISH) for gene copy number and immunohistochemistry (IHC) for protein expression. Prior to *EGFR* mutation analysis samples underwent central histopathological review, and samples were included in the biomarker analysis based on their quality, quantity, type, and tumor content (>100 cells) (Fig. 1). These criteria ensured quality results, reflecting the design of IPASS, determination of differential efficacy in biomarker positive/negative subgroups, limited data at the time regarding the predictive nature of the biomarkers, and extent of validation of the biomarker assays at the time IPASS was conducted (biomarker assays were not validated for cytology samples at that time). This approach provided a definitive answer regarding patients who derived most benefit in the clinical setting. While appropriate to answer the questions posed by the IPASS protocol, the *EGFR* mutation analysis threshold stringency was higher than would be employed for the diagnosis of patients in daily practice. Since IPASS reported, laboratories have gained experience of using existing *EGFR* mutation detection techniques on a spectrum of samples with varying tumor content and sample quality. Small biopsies and cytology samples make up ~30–80% of available diagnostic material, depending on diagnostic practices between different hospitals and countries [12], therefore their successful testing is paramount to ensure this sizeable proportion of patients are given the opportunity to receive optimal treatment. The percentage of mutation testing that occurs using cytology samples can be very variable however, and is currently not consistent across institutions or countries [13]. Smouse et al's retrospective review of *EGFR* sequencing over a two year period at a US hospital noted that only 12/239 (5%) specimens tested for *EGFR* mutation were cytological in origin [13], with focus given to the testing of high-quality tumor tissue samples. Conversely, Hagiwara et al. recently noted that ~40% of samples submitted for *EGFR* mutation testing across three major commercial test centers in Japan were of cytological origin [14], further commenting that this high percentage highlights that cytological samples are indispensable for testing all patients with advanced NSCLC.

The aim of the current study was to investigate whether cytology/histology samples that were not included in the IPASS pre-planned exploratory biomarker analyses could be used successfully to define *EGFR* mutation status and predict which patients were more likely to respond to EGFR-TKI treatment. We describe data generated from pathology review and mutation analysis of the previously unanalyzed histology samples and previously unanalyzed cytology samples, with the aim of testing the outcome of patients with NSCLC as per the study protocol, but by looking at the full spectrum of samples that are available from this population of patients. These data will help to inform the most appropriate thresholds for further trials, as well as the utility of samples received by diagnostic laboratories on a daily basis.



[a]Histology samples that failed pathology review due to insufficient tumor material for biomarker analysis (<100 tumor cells) or poor quality (inadequate fixation or a sample where accurate diagnosis was not possible) and were therefore not included in the main IPASS biomarker analyses. Previously unanalyzed samples included cytology samples and any samples that did not pass pathology review (highlighted in red).
EGFR, epidermal growth factor receptor; IPASS, Iressa Pan-ASia Study.

Fig. 1. The biomarker analysis process.

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2. Materials and methods

2.1. Study design and patients

Full details of IPASS (ClinicalTrials.gov identifier NCT00322452) have been published previously [4,5]. Patients were eligible for inclusion into the study if they had histologically or cytologically confirmed stage IIIB or IV pulmonary adenocarcinoma (including bronchoalveolar carcinoma), were never-smokers (<100 cigarettes in their lifetime) or former light smokers (stopped smoking ≥15 years previously and smoked ≤10 pack-years), and had received no prior chemotherapy, biologic therapy, or immunologic therapy. Patients provided written informed consent with separate consent for the optional assessment of *EGFR* biomarkers. The study protocol was approved by independent ethics committees at each institution. Of 1217 randomized patients, 683 (56%) provided a sample for biomarker analysis. Tumor *EGFR* mutation status was evaluable for 437 patients (261 *EGFR* mutation-positive). Prior to *EGFR* mutation analysis samples underwent central histopathological review; only those considered suitable for the analysis of all exploratory biomarkers, including two methods requiring a specified cell number (*EGFR* gene amplification by FISH requiring 60 cells, and EGFR protein expression by IHC requiring 100 cells, for accurate scoring respectively), were included in the biomarker analysis (sample quality, type, and tumor content [>100 cells]) (Fig. 1). At the time of the original analysis, according to the protocol biomarker analyses were not performed for 215 samples: 116 cytology samples (biomarker analyses had not been validated for this sample type, as previously reported in the appendix of Fukuoka et al. [4]) and 99 histology samples (determined during pathology review not to meet pre-specified biomarker analysis thresholds regarding tumor content [>100 tumor cells] and sample quality/quantity [including samples with inadequate cellular morphology due to poor/inappropriate fixation]). The previously unanalyzed cytology and histology samples are the subject of this additional analysis.

The study was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonisation/Good Clinical Practice, applicable regulatory requirements, and AstraZeneca's policy on bioethics.

2.2. EGFR mutation analyses

EGFR mutation analyses were conducted at two central laboratories (Genzyme, Framingham, MA, USA and AstraZeneca Innovation Center China, Shanghai, China). *EGFR* mutation status of the previously unanalyzed samples was determined by analyzing paraffin-embedded archival histological and cytological cell blocks/smears. Sample tumor content was assessed (histopathological review) prior to categorization based on the number of tumor cells present; 0–9, 10–49, 50–99, and >100 cells. *EGFR* mutations were detected using an amplification mutation refractory system with *EGFR* mutation detection (Qiagen, Manchester, UK), as previously reported for IPASS [5]. Tumors were considered positive if ≥1 of 29 *EGFR* mutations was detected.

2.3. Statistical analyses

Statistical analyses were performed by AstraZeneca. Owing to the small numbers of evaluable cytology and previously unanalyzed histology samples, formal statistical testing was not appropriate. The ORR with exact 95% (Clopper–Pearson) confidence intervals (CIs) was calculated for *EGFR* mutation-positive and -negative cytology samples and *EGFR* mutation-positive and -negative previously unanalyzed histology samples.

Percentage change in tumor size was presented graphically (waterfall plots), with each patient's maximum percentage decrease in tumor size presented as a separate bar (largest increase to largest decrease).

3. Results

3.1. Patients

A total of 215 samples (99 histology; 116 cytology) were available but not analyzed in the main IPASS analysis (Fig. 2). Of the 99 histology samples, 79 (80%) were evaluable for *EGFR* mutations of which 45 (57%) were *EGFR* mutation-positive. Of these 45 patients with *EGFR* mutation-positive tumors, 27 (60%) had received gefitinib and 18 (40%) carboplatin/paclitaxel. Of the 116 cytology samples, 31 (19%) were evaluable for *EGFR* mutation of which nine (29%) were *EGFR* mutation-positive. Of these nine patients with *EGFR* mutation-positive tumors, six (67%) had received gefitinib and three (33%) carboplatin/paclitaxel. A total of 20 histology samples (20%) and 85 cytology samples (73%) were not evaluable for *EGFR* mutation status (insufficient DNA for mutation analysis or no material available for DNA extraction and subsequent analysis).

3.2. Analysis success and tumor cell number: cytology and histology samples that previously failed pathology review

Fig. 3 summarizes the number of evaluable and *EGFR* mutation-positive samples observed, according to tumor cell content. A total of 52 cytology samples (45%) had <100 tumor cells; eleven of these samples provided an evaluable *EGFR* mutation result, of which two (18%) were *EGFR* mutation-positive. A total of 64 cytology samples (55%) had >100 tumor cells; twenty of these samples provided an evaluable *EGFR* mutation result, of which seven (35%) were *EGFR* mutation-positive.

Data from the previously unanalyzed histology samples showed that 73 samples (74%) had <100 tumor cells, with 59 samples providing an evaluable *EGFR* mutation result; thirty (51%) were *EGFR* mutation-positive. A total of 26 histology samples (26%) had >100 tumor cells. These samples had previously been excluded from the main IPASS study on the basis that they did not meet the pre-specified thresholds regarding tumor content and sample quality/quantity (described in Section 2). Twenty samples provided an evaluable *EGFR* mutation result; 15 (75%) were *EGFR* mutation-positive.

In total, therefore, *EGFR* mutation-positive tumors were detected in 54 patients which had previously been described as *EGFR* mutation-unknown.

3.3. Mutation subtype and frequency

Of the *EGFR* mutation-positive cytology samples, 5 (55.6%) were positive for exon 19 deletions and 4 (44.4%) were positive for exon 21 L858R. Of the *EGFR* mutation-positive histology samples, 22 (48.9%) were positive for exon 19 deletions, 18 (40%) for exon 21 L858R, and two (4.4%) for exon 18 G719S/A/C. A total of three samples were identified as having double mutations: two (4.4%) for exon 19 deletions and exon 21 L858R, and one sample (2.2%) for exon 18 G719S/A/C and exon 21 L861Q.

3.4. Efficacy

Data from the previously analyzed samples demonstrated the differential efficacy in terms of ORRs for patients with gefitinib, with 1% of patients ($n = 1/100$) having an objective response in the *EGFR* mutation-negative subgroup, 43% ($n = 167/386$) in the mutation-unknown subgroup, and 71% ($n = 94/132$) in the mutation-positive subgroup [4,5]. Note that in the previous analysis, the *EGFR* mutation-unknown subgroup consisted of 386

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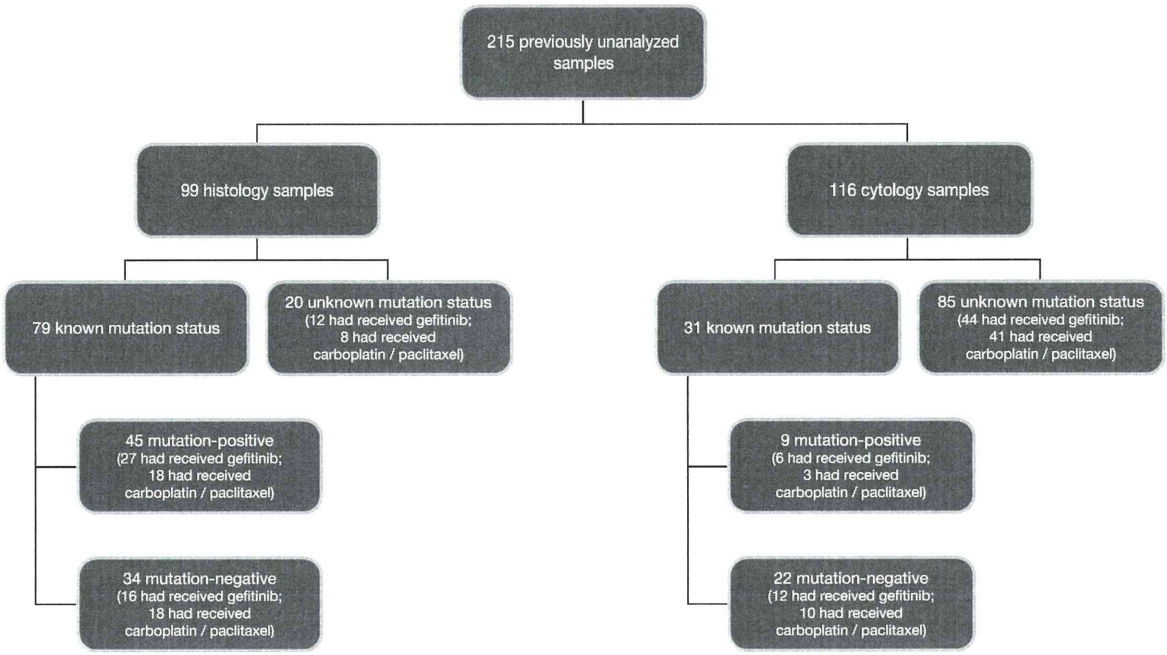
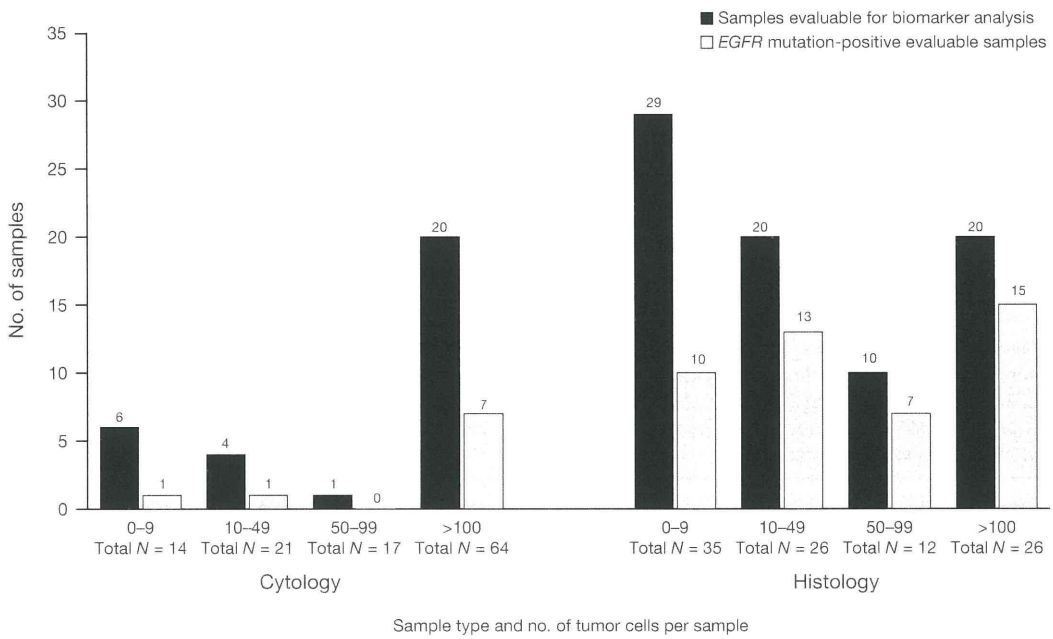


Among the 105 patients for whom tumor *EGFR* mutation status was unknown, the main reasons for unknown *EGFR* mutation status were insufficient DNA for analysis, no material available for analysis, inadequate fixation, and patient diagnosis unable to be confirmed.
EGFR, epidermal growth factor receptor.

Fig. 2. Sample disposition.



EGFR mutations were identified in both the previously unanalyzed histology and cytology samples, with a greater number of histology samples being evaluable for *EGFR* mutation status. *EGFR* mutation pick-up rate in the histology samples was also higher.
EGFR, epidermal growth factor receptor.

Fig. 3. Tumor cell content of the previously unanalyzed histology and cytology samples (intent-to-treat population).

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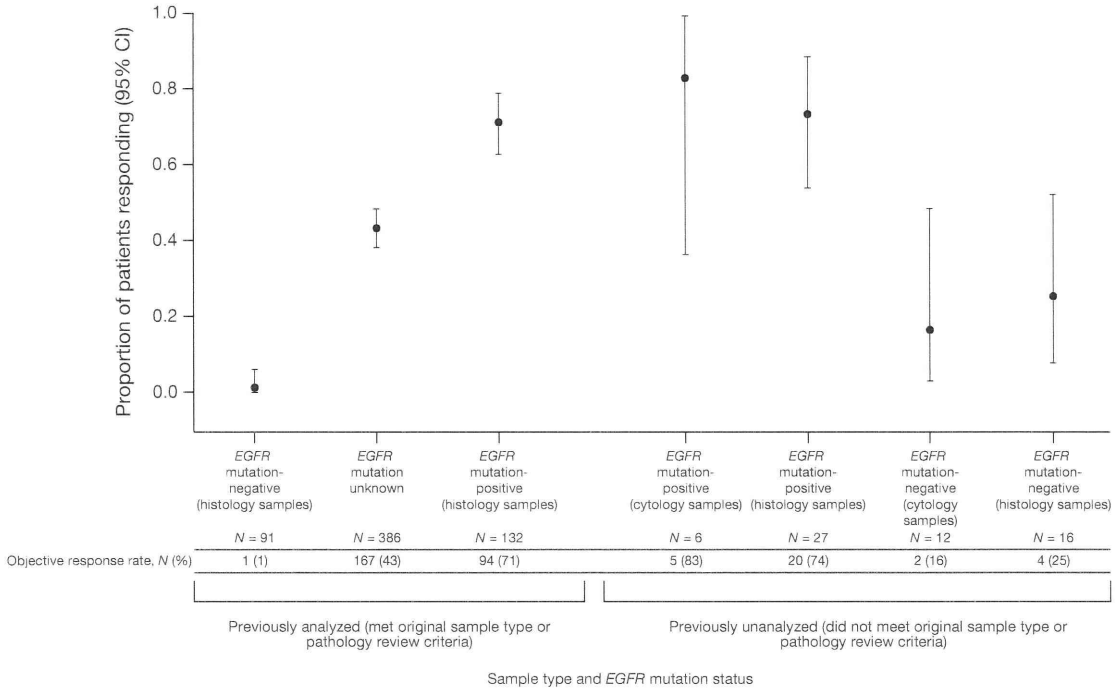

Previously unanalyzed and per-protocol analysis.
Original pathology review criteria included pre-specified thresholds regarding tumor content, sample quality, and sample quantity.
Objective tumor response (complete response or partial response) was determined by the Response Evaluation Criteria in Solid Tumors, version 1.0. Objective response rates were calculated as the percentage of the total number of patients analyzed whose tumors had a confirmed overall response of complete response or partial response.
CI, confidence interval; *EGFR*, epidermal growth factor receptor.

Fig. 4. Objective response rate for patients treated with gefitinib by sample type and *EGFR* mutation status (intent-to-treat population).

patients, including 61 patients described as not previously analyzed and who are described here.

Fig. 4 summarizes the ORR in the previously unanalyzed cytology and histology samples by *EGFR* mutation status for patients with gefitinib. The ORR in the *EGFR* mutation-positive subgroups by cytology and previously unanalyzed histology samples are consistent with the data from the previously determined *EGFR* mutation-positive subgroups: *EGFR* mutation-positive on the basis of cytology ORR 83% ($n = 5/6$), previously unanalyzed histology sample 74% ($n = 20/27$) versus 71% in the previous analysis. The ORR in the *EGFR* mutation-negative subgroups by cytology and previously unanalyzed histology samples are higher than those observed in the previously determined *EGFR* mutation-negative subgroups: *EGFR* mutation-negative on the basis of cytology 16% ($n = 2/12$), previously unanalyzed histology sample 25% ($n = 4/16$) versus 1% in the previous analysis.

Tumor size reduction (percentage change from baseline) with gefitinib in the previously unanalyzed cytology and histology samples appears to be consistent with previously analyzed histology samples, for both *EGFR* mutation-positive (Fig. 5a and b) and -negative samples (Fig. 5d and e). The *EGFR* mutation-positive and -negative tumors from the updated analysis are evenly distributed throughout the waterfall plots of the previously analyzed histology samples (Fig. 5c and f, respectively). Maximum percentage change in tumor size from baseline for patients whose tumors were of unknown *EGFR* mutation status is shown in Fig. 6a (including previously analyzed samples, and cytology and low tumor content samples), Fig. 6b (previously unanalyzed samples highlighting those cytology and low tumor content tumor samples subsequently found to be *EGFR* mutation-positive), and Fig. 6c (previously unanalyzed samples highlighting those cytology and low tumor content tumor samples subsequently found to be *EGFR* mutation-negative).

4. Discussion and conclusions

The results of IPASS clearly demonstrated the differential efficacy of EGFR-TKIs in the *EGFR* mutation-positive, -negative, and -unknown subgroups [4,5]. EGFR-TKIs are now recommended for the treatment of patients with *EGFR* mutation-positive tumors [15]. As a result of available data, accurate identification of patients who might benefit from EGFR-TKI therapy has become an important step in the treatment-decision pathway for advanced NSCLC [16].

This study shows that both histology and cytology samples used to diagnose NSCLC are suitable for the detection of *EGFR* mutations. This study demonstrates that where an *EGFR* mutation-positive result is observed, EGFR-TKI efficacy is consistent with that observed in the sample analysis according to the protocol, albeit with wider ORR CIs due to sample number. In both the cytology and previously unanalyzed histology subgroups, a higher response rate was observed in samples in which no *EGFR* mutation was detected compared with the 1% response rate in the previously analyzed histology samples in which no mutation was detected. While the *EGFR* mutation frequency is as expected in the previously unanalyzed histology samples, it was lower than expected in the cytology samples. Taken together, these two observations demonstrate that there are likely to be a number of false-negative results within the *EGFR* mutation-negative (or *EGFR* mutation-not-detected) subgroups in these previously unanalyzed samples, showing that the *EGFR* mutation-negative results are less robust than in the previously analyzed samples of good quality/quantity. This study therefore demonstrates that while high quality and high tumor content samples should be obtained and tested where possible, it is feasible to use low tumor content or cytology samples if these are the only sample available from the initial diagnosis of advanced NSCLC. Additionally, feedback from pathologists and molecular biologists on sample quality would help to minimize

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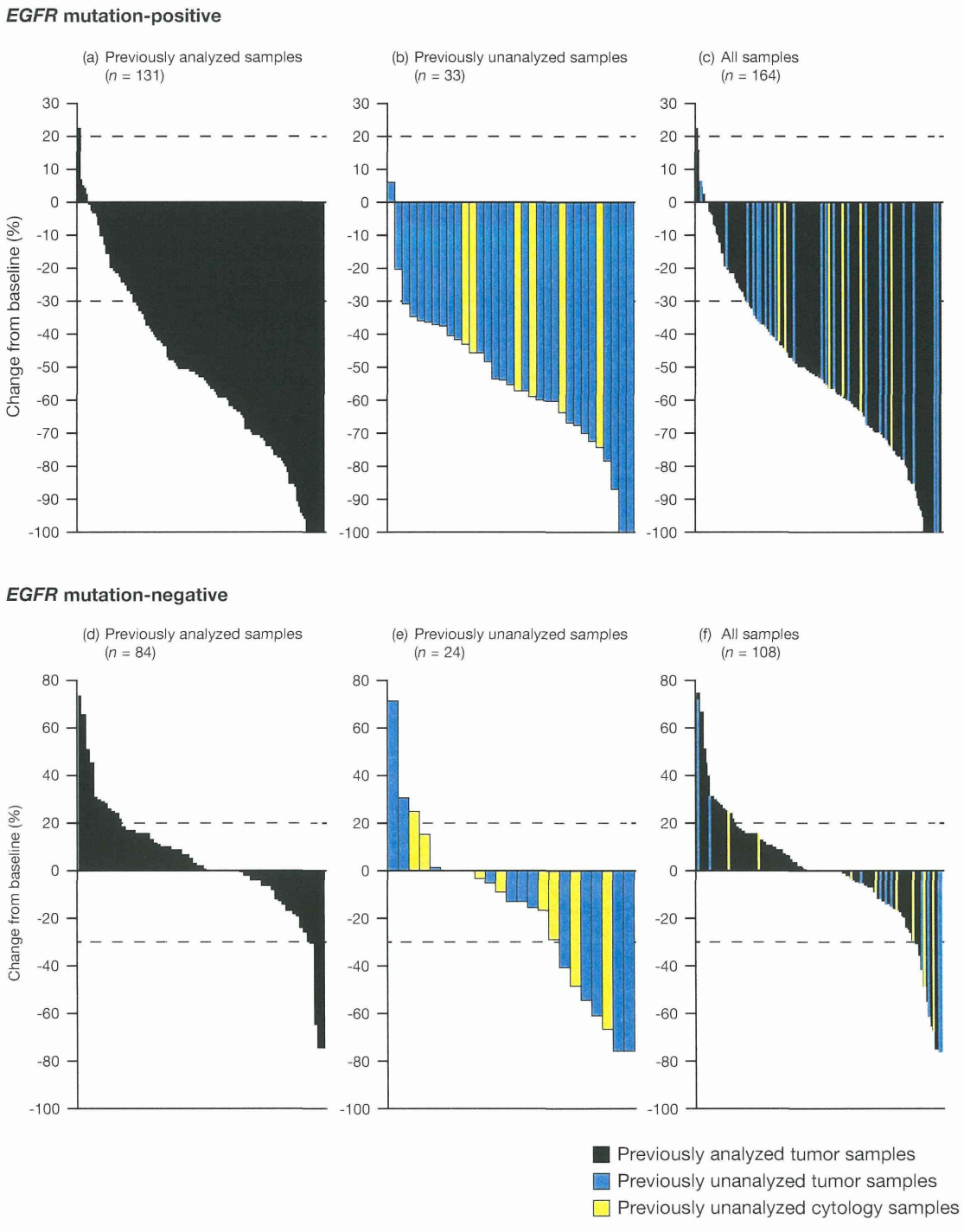

The horizontal dashed lines at -30% and +20% represent the percentage change required for a response or progression of target lesions, respectively, according to Response Evaluation Criteria In Solid Tumors, version 1.0.
Only patients with a baseline and one evaluable post-baseline target lesion assessment are included. Plots do not include assessment of non-target or new lesions.

EGFR, epidermal growth factor receptor.

Fig. 5. Waterfall plots for maximum percentage change in tumor size from baseline in patients with *EGFR* mutation-positive tumors treated with gefitinib from (a) previously analyzed samples, (b) previously unanalyzed samples, and (c) all analyzed samples; and *EGFR* mutation-negative tumors treated with gefitinib from (d) previously analyzed samples, (e) previously unanalyzed samples, and (f) all analyzed samples.

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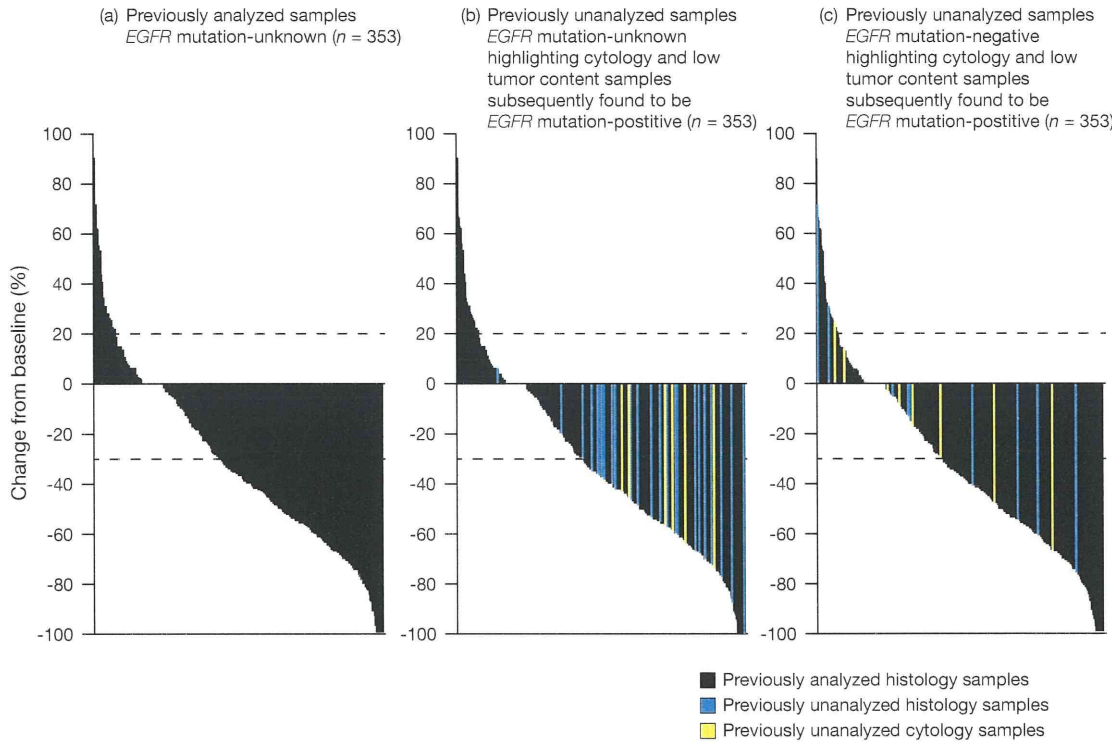

The horizontal dashed line at 30% shrinkage represents the percentage change required for a response of target lesions according to Response Evaluation Criteria In Solid Tumors, version 1.0.
Only patients with a baseline and one evaluable post-baseline target lesion assessment are included. Plots do not include assessment of non-target or new lesions.

EGFR, epidermal growth factor receptor.

Fig. 6. Waterfall plots for maximum percentage change in tumor size from baseline in patients with tumors of unknown *EGFR* mutation status treated with gefitinib from (a) previously analyzed samples, (b) previously unanalyzed samples with *EGFR* mutation-positives from updated analysis, and (c) previously unanalyzed samples with *EGFR* mutation-negatives from updated analysis.

the costs of repeat testing and optimize the process of obtaining a quality result that the physician can take into consideration when making a treatment decision.

The importance of ensuring that samples are of sufficient quality/quantity has been confirmed in this study. The *EGFR* mutation frequency observed in the cytology samples implies that the pre-specified tumor content of 100 cells is still relevant within the clinical setting in order to avoid the issue of false-negative results in this sample type. In contrast, these data suggest that for histology sample analysis, it may be possible to reduce the criteria.

Several groups have released recommendations for *EGFR* mutation testing practices which include guidance on good quality/quantity samples, but little guidance on how laboratories should deal with low tumor content or cytology samples [17–20]. Any samples used for diagnosis of NSCLC (e.g. biopsy, resection, cytology) should be tested for *EGFR* mutation status provided the laboratory performing the analysis is confident in the result. This confidence will depend on the method used, laboratory expertise, and the quality/quantity of the samples, typically those that contain sufficient tumor material to obtain an accurate result, regardless of sample source. Testing of samples judged to be of low quality or low tumor content should be carried out using sensitive testing methods with or without a technique such as Laser Capture Microdissection (LCM), to enrich for the tumor cells. This technique was not attempted in IPASS, because while the technology is available in some institutions, it is not widely available and therefore not possible for all routine *EGFR* testing labs to employ. The Molecular Assays in NSCLC Working Group highlighted that LCM may be used to facilitate accurate test results by increasing the ratio of tumor to normal tissue, which is particularly important for techniques such as direct sequencing, which requires samples with ≥50–70% tumor cells for analysis [17]. However, the Working Group also noted that LCM can be laborious, and is unlikely to be acceptable for routine clinical sample analysis.

This analysis of previously unanalyzed samples from IPASS has shown that NSCLC samples of either low tumor cell content or cytological origin are suitable for the detection of *EGFR* mutation-positive disease. While consideration should be given to the individual capabilities of diagnostic laboratories, the testing of these additional samples may lead to an increase in the number of successful mutation results, enabling a greater number of patients to be accurately diagnosed, and receive the most effective and personalized therapy.

Role of the funding source

This work was supported by AstraZeneca, UK.

Conflict of interest statement

J.C.-H. Yang has received advisory fees from AstraZeneca, Roche, Genentech, Pfizer, and Clovis, and has been an uncompensated advisor to Boehringer Ingelheim and Eli Lilly. Y.-L. Wu and K. Nakagawa have received speaker fees from AstraZeneca.

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G. McWalter and R. McCormack are employees of AstraZeneca and hold shares in AstraZeneca. T.S. Mok has received research funding from AstraZeneca and advisory fees from AstraZeneca, Roche, Eli Lilly, Boehringer Ingelheim, Merck Serono, and Pfizer. M. Fukuoka, N. Saijo, V. Chan, and J. Kurnianda have no conflicts of interest to disclose.

Acknowledgements

The authors would like to thank the patients and investigators for their participation in the IPASS study. Sample analysis was performed by Dr Guanshan Zhu, Dr Li Zheng, and Dr Peter Lu at Innovation Center China (China cohort) and Genzyme genetics (non-China samples). Statistical analysis was performed by Dr Rosie Taylor from AstraZeneca, UK. Editing support funded by AstraZeneca was provided by Sarah Lewis, from Complete Medical Communications.

References

[1] Azzoli CG, Baker Jr S, Temin S, Pao W, Aliff T, Brahmer J, et al. American Society of Clinical Oncology Clinical Practice Guideline update on chemotherapy for stage IV non-small-cell lung cancer. J Clin Oncol 2009;27:6251–66.
[2] Azzoli CG, Temin S, Aliff T, Baker Jr S, Brahmer J, Johnson DH, et al. 2011 focused update of 2009 American Society of Clinical Oncology Clinical Practice Guideline update on chemotherapy for stage IV non-small-cell lung cancer. J Clin Oncol 2011;29:3825–31.
[3] Kris MG, Johnson BE, Kwiatkowski DJ, Lafrate AJ, Witsuba II, Aronson SL, et al. Identification of driver mutations in tumor specimens from 1,000 patients with lung adenocarcinoma: the NCI's lung cancer mutation consortium (LCMC). J Clin Oncol 2011;29(Suppl.) [abstract CRA7506].
[4] Fukuoka M, Wu Y-L, Thongprasert S, Sunpaweravong P, Leong S-S, Sriuranpong V, et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol 2011;29:2866–74.
[5] Mok TS, Wu Y-L, Thongprasert S, Yang C-H, Chu D-T, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947–57.
[6] Han JY, Park K, Kim SW, Lee DH, Kim HY, Kim HT, et al. First-SIGNAL: first-line single-agent Iressa versus gemcitabine and cisplatin trial in never-smokers with adenocarcinoma of the lung. J Clin Oncol 2012;30:1122–8.
[7] Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380–8.
[8] Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010;11:121–8.
[9] Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239–46.
[10] Sequist LV, Yang JCH, Yamamoto N, O'Byrne KJ, Hirsh V, Mok TSK, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol 2013:3327–34, http://dx.doi.org/10.1200/JCO.2012.44.2806.
[11] Zhou C, Wu Y-L, Chen G, Feng J, Liu X-Q, Wang C, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced *EGFR* mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2011;12:735–42.
[12] Hlinkova K, Babal P, Berzinec P, Majer I, Mikle-Barathova Z, Piackova B, et al. Evaluation of 2-year experience with EGFR mutation analysis of small diagnostic samples. Diagn Mol Pathol 2013;22:70–5.
[13] Smouse JH, Cibas ES, Janne PA, Joshi VA, Zou KH, Lindeman NI. EGFR mutations are detected comparably in cytologic and surgical pathology specimens of nonsmall cell lung cancer. Cancer 2009;117:67–72.
[14] Hagiwara K, Kobayashi K. Importance of the cytological samples for the epidermal growth factor receptor gene mutation test for non-small cell lung cancer. Cancer Sci 2013;104:291–7.
[15] D'Addario G, Früh M, Reck M, Baumann P, Klepetko W, Felip E. Metastatic non-small-cell lung cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010;21(Suppl. 5):v116–9.
[16] Keedy VL, Temin S, Somerfield MR, Beasley MB, Johnson DH, McShane LM, et al. American Society of Clinical Oncology provisional clinical opinion: epidermal growth factor receptor (EGFR) mutation testing for patients with advanced non-small-cell lung cancer considering first-line EGFR tyrosine kinase inhibitor therapy. J Clin Oncol 2011;29:2121–7.
[17] Eberhard DA, Giaccone G, Johnson BE. Biomarkers of response to epidermal growth factor receptor inhibitors in non-small-cell lung cancer working group: standardization for use in the clinical trial setting. J Clin Oncol 2008;26:983–94.
[18] Marchetti A, Normanno N, AIOM-SIAPEC-IAP, Pinto C, Taddei GL, Adamo V, et al. Recommendations for mutational analysis of EGFR in lung carcinoma. Pathologica 2010;102:119–22.
[19] Pirker R, Herth FJ, Kerr KM, Filipits M, Taron M, Gandara D, et al. Consensus for EGFR mutation testing in non-small cell lung cancer: results from a European workshop. J Thorac Oncol 2010;5:1706–13.
[20] Salto-Tellez M, Tsao MS, Shih JY, Thongprasert S, Lu S, Chang GC, et al. Clinical and testing protocols for the analysis of epidermal growth factor receptor mutations in East Asian patients with non-small cell lung cancer: a combined clinical-molecular pathological approach. J Thorac Oncol 2011;6:1663–9.

Please cite this article in press as: Yang JC-H, et al. Epidermal growth factor receptor mutation analysis in previously unanalyzed histology samples and cytology samples from the phase III Iressa Pan-ASia Study (IPASS). Lung Cancer (2013), http://dx.doi.org/10.1016/j.lungcan.2013.11.021

Invest New Drugs (2013) 31:1568–1572
DOI 10.1007/s10637-013-0014-0

PHASE II STUDIES

A phase II study of cisplatin /S-1 in patients with carcinomas of unknown primary site

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Received: 1 July 2013 / Accepted: 13 August 2013 / Published online: 24 August 2013

Summary *Background* Carcinomas of unknown primary site (CUPs) are heterogeneous tumors associated with a poor prognosis. This phase II trial was designed to evaluate the efficacy and safety of a novel combination chemotherapy of S-1 and cisplatin (CDDP) in patients with CUP. *Patients and Methods* Patients with previously untreated CUPs were eligible for this trial. The treatment schedule consisted of oral S-1 (40 mg/m^2) twice a day on days 1–21, and intravenous CDDP (60 mg/m^2) on day 8. This schedule was repeated every 5 weeks. *Results* A total of 46 patients were enrolled. The overall response rate and the disease control rate were 41.3 % and 80.4 %, respectively. The median overall survival time was 17.4 months. Grade 3/4 neutropenia, thrombocytopenia, and febrile neutropenia occurred in 28.3 %, 13.0 %, and 2.2 % of the patients, respectively. *Conclusion* CDDP plus S-1 combination chemotherapy is well tolerated and active first-line empiric therapies for patients with CUP.

Key words Cisplatin · S-1 · Chemotherapy · Carcinoma of unknown primary site

Clinical study: identifier UMINC000000271

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Introduction

Carcinomas of unknown primary site (CUPs) represent a group of heterogeneous tumors that has no identifiable origin. Despite advances in tumor pathology and imaging techniques, such as positron emission tomography, CUPs account for about 5 % of all cancers [1]. Several clinicopathological subsets with favorable prognosis have been identified. However, most patients do not fit into any of these subsets. Because the prognosis of CUP is generally poor, with a median overall survival time (OS) of 6–13 months, the benefit of chemotherapy compared with best supportive care is still unclear [2]. During the past 3 decades, some phase II trials of platinum-based combination regimens containing newer cytotoxic agents (taxanes, gemcitabine, and irinotecan) resulted in response rates of 30 %–40 % and median survivals of 8–11 months [3–9].

S-1 is a new oral fluoropyrimidine agent designed to enhance antitumor activity and to reduce gastrointestinal toxicity through the combined use of an oral fluoropyrimidine agent (tegafur), a dihydrophyrimidine dehydrogenase inhibitor (5-chloro-2,4-dihydroxypyridine), and an orotate phosphoribosyl transferase inhibitor [10]. Treatment with the single agent S-1 results in response rates of 49 % in advanced gastric cancer patients [11] and 35 % in metastatic colorectal cancer patients [12]. Good results have also been reported in breast cancer, lung cancer, pancreatic cancer [13–15], and head and neck cancer. Thus, S-1 has a broad spectrum of clinical activity in

solid tumors. Moreover, preclinical studies showed a synergistic effect between S-1 and cisplatin (CDDP) [16]. In the hopes of developing a more safe and effective therapy, we conducted a phase II study of this novel combination chemotherapy of CDDP and S-1 in patients with CUP.

Patients and methods

This nonrandomized phase II trial was initiated in October 2005 and performed at multiple institutions.

Patient eligibility Patients eligible for this trial were required to have a previously untreated, histologically or cytologically documented CUP. For purposes of this trial, patients were considered to have CUP if no primary site was evident after an evaluation including medical history; physical examination; complete blood counts; chemistry profile; computed tomography (CT) scan of the chest, abdomen, and pelvis; and directed radiologic or endoscopic workup of symptomatic areas. Histological examination which includes standard immunohistochemistry (IHC) inspection for diagnosis as CUP was required. Additional eligibility criteria included measurable disease, Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2, age of 20 years or older, adequate hematologic, renal, and hepatic functions. Patients known to have good prognostic features were excluded. These subsets included: 1) patients with a single metastasis; 2) women with adenocarcinoma isolated to axillary lymph nodes; 3) patients with squamous carcinoma limited to cervical or inguinal nodes; 4) patients with features of neuroendocrine or extragonadal germ cell tumor; 5) men with high levels of serum prostate-specific antigen (PSA) or positive for PSA by immunohistochemistry; and 6) women with peritoneal carcinomatosis. Patients were excluded if they had symptomatic brain metastases. Additional exclusion criteria included concomitant serious diseases and pregnant or lactating females. Written informed consent was obtained from all patients, and the protocol was approved by the institutional ethics committee of each of the participating institutions.

Treatment S-1 was administered orally, 40 mg/m^2 twice a day after meals between days 1 and 21. CDDP (60 mg/m^2) was administered intravenously on day 8 when patients were hydrated with enough infusion. The oral dose of S-1 for each patient was assigned based on the body surface area. The 3 doses administered were 40 mg (body surface area$<$1.25 m^2), 50 mg (1.25 m^2$\leq$body surface area$<$1.50 m^2), and 60 mg (body surface area$\geq$1.50 m^2). Supportive care, which included adequate hydration and antiemetics, was provided at the discretion of the patient's physician and respective institution. If laboratory variables were not met eligibility criteria for the CDDP administration on day 8, administration of CDDP were withheld until the abnormality had resolved. If there was no resolution of abnormality within day15, the patient skipped CDDP administration. The treatment regimen was repeated every 5 weeks for at least 3 cycles unless disease progression or unacceptable toxicity occurred. The doses of S-1 were reduced in the event of any of the following toxicities during the previous treatment cycle: grade 4 hematological toxicity, or grade 3 or higher nonhematological toxicity. For the subsequent treatment courses, S-1 was reduced from 60, 50, or 40 mg twice daily to 50, 40, or 25 mg twice daily, respectively. If a rest period of more than 28 days was required, then the patient stopped the protocol treatment.

Evaluation of response and toxicity All eligible patients were considered assessable for response and toxicity. The response was evaluated in accordance with the Response Evaluation Criteria in Solid Tumors [17]. The response was confirmed for at least 4 weeks (for complete response [CR] or a partial response [PR]) after it was first documented or 6 weeks (for stable disease [SD]) after the start of therapy. Progression-free survival (PFS) was defined as the time from registration until objective tumor progression or death. OS was defined as the time from registration until to death from any cause. Adverse

Table 1 Patient characteristics(N=46)

Characterisitic	No. of Patients	(%)
Median age, years (range)	63	(31–84)
Gender		
Male	25	(54.3)
Female	21	(45.7)
ECOG performance status		
0	4	(8.7)
1	37	(80.4)
2	5	(10.9)
Histology		
Adenocarcinoma	23	(50.0)
Poorly differentiated adenocarcinoma	3	(6.5)
Poorly differentiated carcinoma	3	(6.5)
Squamous carcinoma	14	(30.4)
Other	3	(6.5)
Dominant site of disease		
Lymph nodes	40	(87.0)
Only nodal disease	18	(39.1)
Bones	10	(21.7)
Lung	8	(17.4)
Liver	5	(10.9)
Adrenal	3	(6.5)
Pancreas	3	(6.5)
Others	10	(21.7)

ECOG (Eastern Cooperative Oncology Group)

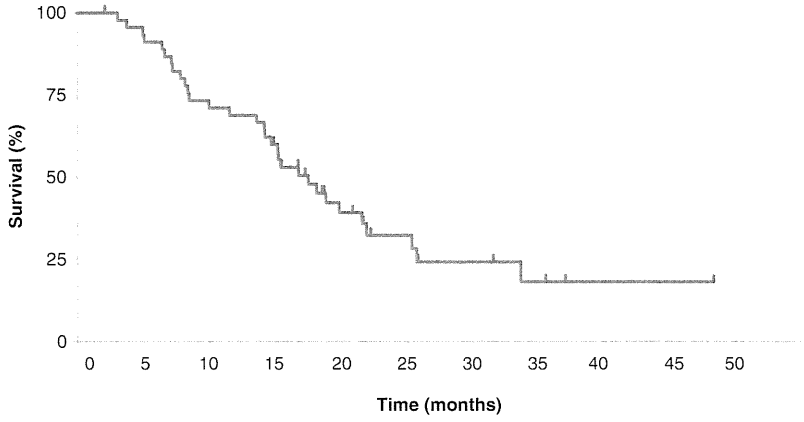

Fig. 1 Kaplan-Meier plot for overall survival (N=46)

events were graded according to National Cancer Institute Common terminology criteria for Adverse Events, version 3.0.

Statistical analysis The primary end point of this study was the response rate, and the secondary endpoints were toxicity, OS, and 1-year survival rate. In previous reports with first-line therapy of patients with CUP, the range of response rate was 25 %–55 %. Therefore, the number of patients to be enrolled in this study was calculated as 45, which was the number required to refute the assumption that the 95 % confidence interval (95 % CI) would be 25 % under conditions of α=0.05 and β=0.2, while assuming an expected response rate of 45 %. OS and PFS were estimated by the Kaplan–Meier method.

Results

Patient characteristics Between October 2005 and September 2009, 46 patients were enrolled on this clinical trial and were treated with a combination of S-1 and CDDP. The patient characteristics are summarized in Table 1. The median age was 63 years (range, 31–84 years). Most of the patients had an ECOG performance status (PS) of 0–1, and only 5 (10.9 %) patients had a PS of 2. 25 patients were male. 23 patients had adenocarcinoma, 14 had squamous cell carcinoma, 3 had poorly differentiated carcinoma, and 3 had poorly differentiated adenocarcinoma. 18 patients presented with lymph nodes metastasis only.

Treatment delivery The median number of treatment courses of the S-1 and CDDP regimen received by patients on this trial was 4 (range 1–10). 31 patients (67.4 %) received at least 3 treatment courses. 15 patients withdrew treatment prior to completing 3 courses for the following reasons: tumor progression in 11 patients, treatment-related toxicity in 1 patient, and patient's refusal in 3 patients. In accordance with the study protocol, dose reductions were necessary in 7 patients. CDDP was skipped in 6 patients because of following reasons: tumor progression in 1 patient, hematologic toxicities in 4 patients, and nausea in 1 patient.

Efficacy The overall response rate was 41.3 % (95 % CI, 27.0 to 56.8). The disease control rate (DCR; CR+PR+SD) was

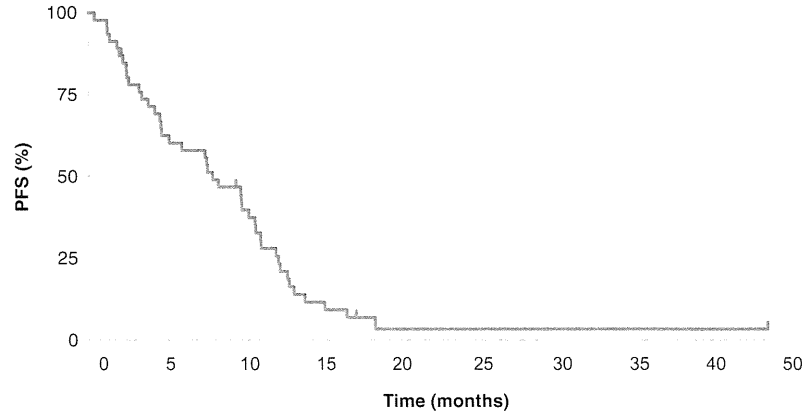

Fig. 2 Kaplan-Meier plot for progression-free survival (N=46)